



Two new species segregated from *Hymenophyllum tunbrigense* (Hymenophyllaceae) in southern South America, based on morphological, anatomical, molecular and distributional evidence

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Abstract

The cosmopolitan species *Hymenophyllum tunbrigense* was traditionally represented in southern South America by two allopatric varieties: *H. tunbrigense* var. *tunbrigense* in the Andean Patagonian forests of Argentina and Chile, and *H. tunbrigense* var. *cordobense*, an endemic taxon restricted to the mountain system of central and north-western Argentina. Given the diagnosable differences between these two taxa, and between these taxa and the European and African entity, based on morphological, anatomical, molecular, ecological and distributional evidence, we exclude *H. tunbrigense* for Southern Cone, propose to revalidate the name *Hymenophyllum asperulum* for the species present in the Magellanic and Valdivian forests and elevate *H. tunbrigense* var. *cordobense* to species rank. We consider these two taxa as endemic species, closely related to the widespread *H. tunbrigense*. We also cite *Hymenophyllum cordobense* for first time for the flora of Bolivia.

Key words: *Hymenophyllum asperulum*, *H. cordobense*, Pampean mountains, Endemism, Taxonomy, Yungas, Austral Andes

Resumen

La especie cosmopolita *Hymenophyllum tunbrigense* ha sido tradicionalmente considerada para el sur de Sudamérica con dos variedades alopatricas: *H. tunbrigense* var. *tunbrigense* en los bosques andino-patagónicos de Argentina y Chile, e *H. tunbrigense* var. *cordobense*, un endemismo restringido a los sistemas montañosos del centro y noroeste de Argentina. Dadas las diferencias diagnósticas entre estos dos taxones, y considerando las diferencias de éstos con la entidad de África y Europa, basándonos en las evidencias morfológicas, anatómicas, moleculares, ecológicas y distribucionales, excluimos a *H. tunbrigense* del Cono Sur, proponemos revalidar el nombre *Hymenophyllum asperulum* para la especie presente en los bosques valdivianos y magallánicos y elevar al rango de especie a *Hymenophyllum tunbrigense* var. *cordobense*. Consideramos a estas dos entidades como especies endémicas, estrechamente relacionadas con la ampliamente distribuida *H. tunbrigense*. Se cita además a *H. cordobense* por primera vez para la flora de Bolivia.

Introduction

Hymenophyllum Smith (1793: 418) comprises about 250 species (PPG I 2016) distributed pantropically and in temperate areas, confined to very humid places. *Hymenophyllum tunbrigense* (L.) Smith (1793:418), the type species of the genus, was historically treated in a very broad sense including specimens of the Old and New World (Hooker 1844, Diem & Lichtenstein 1959, Pacheco 1995, Mickel & Smith 2004, Larsen *et al.*, 2013), based on general morphology and a supposed cosmopolitan distribution. The phylogenetic study by Hennequin *et al.* (2010) showed that the specimens from Africa, South America and Europe were closely related, and suggested that the species indeed does have a cosmopolitan distribution. However, in the molecular study carried out by Hennequin *et al.* (2010) only one specimen for each continent was analysed and no morphological studies were included.

In floristic treatments for the Southern Cone of South America, *Hymenophyllum tunbrigense* has traditionally been considered to comprise two allopatric varieties (de la Sota 1977, 1998, Ponce & de la Sota 2008, Larsen 2014): *Hymenophyllum tunbrigense* var. *tunbrigense* in the Andean Patagonian forests of Argentina and Chile (0–800 m) and *H. tunbrigense* var. *cordobense* Hieronymus (1896: 361), which is restricted to the mountainous system of central and north-western Argentina (1200–2400 m). The two varieties are separated by 1300 km of arid environments, called South American Transition Zone (Morrone 2006, 2015). Diem & Lichtenstein (1959) also included *H. tunbrigense* var. *asperulum* from southern Argentina and Chile, which are smaller plants with entire indusia.

Hieronymus (1896) described *H. tunbrigense* var. *cordobense* based on specimens from Córdoba province in central Argentina. The variety was distinguished from typical *H. tunbrigense* by differences in the shape and the width of the lamina, the relative position of the pinnae (de la Sota 1973, 1977, Arana 2012, Larsen 2014), narrower segments and a few and short hairs over the axes (Diem & Lichtenstein 1959). This local variety inhabits the cloud forest on the western slopes of the Andes, starting at 1200 m altitude, in the biogeographic province of austral Yungas of Argentina (Cabrera & Willink 1973, Morrone 2006, 2014), and also reaches the mountain systems of Central Argentina called *Sierras Pampeanas centrales* (Central Pampean Ranges), in the Chaco biogeographic province (Arana & Bianco 2011).

Diem and Lichtenstein (1959) decided to include specimens from Patagonian forests in *Hymenophyllum tunbrigense* var. *tunbrigense*, because they regarded them to be most similar to the European and African form. However they mentioned some differences: they are smaller; the leaf is more rounded and less divided, less toothed and sometimes with entire indusia. In the same way, var. *asperulum* was defined as differing from the European form, with less divided laminae and entire indusia. This last variety was synonymized with the typical variety by Larsen *et al.* (2013), because they considered the differences, based mainly on the size of the plants, as insignificant.

In this paper we reevaluate *Hymenophyllum tunbrigense* regarding the status of the intraspecific variability in southern South America, including phylogenetic, morphologic and anatomic evidence, ecologic and distributional information, and complete descriptions of the taxa.

Materials and methods

Morphology and Anatomy

Morphological and anatomical observations were performed based on the study of fresh material and herbarium specimens, including types. Collections of the herbaria BA, BAB, BCRU, CONC, CORD, CTES, JUA, LIL, LP, LPB, MCNS, RCVC, RIOC and SI (herbarium acronyms follow Thiers 2015) were consulted. European and South African specimens observed are listed in Appendix 1. The ecological parameters and the field observations were taken during exploration trips to the Argentinean provinces of Chubut, Córdoba, Jujuy, Neuquén, Río Negro, Salta, Santa Cruz and Tucumán from 2008 to 2016.

The samples were placed in FAA (95% ethanol: glacial acetic acid: 37–40% formaldehyde: water; 50:5:10:35, v/v). The dehydration of the samples was done according to the procedures outlined by Johansen (1940), using graduated solutions of ethanol and xylene. In order to prepare permanent slides, fully infiltrated tissues were embedded in Histowax (highly purified paraffin wax blended with polymer additives). The samples were triple-stained with hematoxylin, safranin O and fast green FCF as described by Johansen (1940). To analyse the histological preparations a standard Zeiss Model 16 microscope was used and photomicrographs were taken with an equipment of image capture and digitalization AxioVision 4.3 with camera AxioCam HRc, in a Zeiss Axiophot microscope. The one-cell thick lamina allows the observation of laminar cellular characters without section.

For morphological and anatomical comparisons, only fertile and mature fronds were chosen to take measures. The anatomical analysis was made only on the South American specimens.

Molecular Phylogeny

A molecular phylogeny using four chloroplast markers (*rbcl*, *rbcl-accD*, *rps4-trnS* and *trnG-R*; see Hennequin *et al.* 2010), was constructed including almost all taxa of subgenus “*Hymenophyllum*” (Hennequin *et al.* 2010, fig 4, grey square), three samples of *H. tunbrigense* var. *cordobense* and two samples of *H. tunbrigense* var. *tunbrigense* from Argentina and Chile. The specimens examined identified with an asterisk (*) were sequenced for this work. A total of 36 samples were used in the phylogenetic analysis (appendix 2). *H. villosum* Colenso (1845: 185), *H. cruentum* Cavanilles (1802: 275), *H. demissum* (G. Forster 1786: 85) Swartz (1801: 100), *H. hirsutum* (Linnaeus 1753: 1098)

Swartz (1801: 99) and *H. apiculatum* Mett. ex Kuhn (1868: 391) were selected as outgroup. Total DNA of species was extracted from silica gel-dried tissue, following a modified total CTAB method used by Doyle & Doyle (1987). Amplifications were carried out following either Hennequin *et al.* (2003) or Hennequin *et al.* (2010). Sequencing was performed by Macrogen, Inc. (Seoul, Korea), using the ABI PRISM Big Dye Terminator Cycle Sequencing Kits with Ampli Taq DNA polymerase (Applied Biosystems, Seoul, Korea) following the protocols supplied by the manufacturer. Alignments were generated with Clustal X v. 2 (Larkin *et al.* 2007) under the default settings. The alignments obtained were then checked and improved manually where necessary by visual refinement using the program MEGA v.6 (Tamura *et al.* 2013).

Bayesian Inference (BI) analyses were performed using the CIPRES Science Gateway V.3.3 (Miller *et al.* 2010). The dataset was partitioned according to marker, and the best-fit model of nucleotide substitution for each marker was identified using the Akaike information criterion (AIC) implemented in jModeltest 2.1.3 (Darriba *et al.* 2012) (GTR+I+G for all markers). The BI was carried out with Mr Bayes v.3.2.1 (Ronquist *et al.* 2012) implementing two parallel runs of four simultaneous Markov chains for 10 million generations, sampling every 1000 generations and using the default parameters. The convergence of the runs was assessed by checking the status of parameters in Tracer 1.5 (Rambaut & Drummond, 2007) to ensure the stationarity of each run. Likelihoods of the trees produced by each run were analyzed graphically using Tracer v.1.4 (Rambaut & Drummond, 2007) and, after discarding the initial 2500 trees of each run as burn-in (25%), the remaining trees were summarized in a 50% majority consensus tree including the posterior probabilities as branch support estimates.

Results and discussion

Morphological and anatomical analyses

The European specimens of *Hymenophyllum tunbrigense* can be differentiated from the South American specimens because the Old World specimens are larger plants with leaves more divided and indusia notably erose to irregularly dentate, versus smaller plants, less divided leaves and entire to slightly denticulate or erose indusia in the South American specimens. Even though we could not obtain herbarium specimens from Central America, it should be noted that the specimens from this region described by Mickel & Smith (2004) are morphologically similar to the European *H. tunbrigense*. This was confirmed by the study of images of herbarium specimens from Central America. Therefore, considering these morphological features, we propose to segregate the entities from South America as different taxa. Also, due to similarities between specimens from North and Central America and Europe, we included both within *H. tunbrigense* *sensu lato*, although their phylogenetic position are not fully solved here and should eventually be tested in additional studies.

In regard to the South American specimens, we propose to revalidate the available name *Hymenophyllum asperulum* for the Patagonia forest species, and the name *H. cordobense* to the northwestern Argentina and Bolivia species. These two taxa share some features like the denticulate laminar margin, the presence of simple, multicellular, reddish hairs on the rachis and veins, and sori with a thickened base located on short acroscopic segments of the pinnae. However in this study we observed that *H. cordobense* also differs from *H. asperulum* by several characters: narrower rhizomes, narrower laminae ovate-lanceolate to linear-lanceolate in outline, attenuate apex, remote middle pinnae (never overlapping), narrower segments, and ovate sori with the base not immersed in the lamina (Fig. 1).

Additionally, the anatomical analysis shows that *H. cordobense* is distinguished from *H. asperulum* by the shape and cell number of glandular hairs of the rachis and veins that are always bicellular, with the apical cell ellipsoid, 2–3 times longer than the basal one (vs. glandular hairs capitate, the apical cell spherical in *H. asperulum*); with scarce simple longer hairs (vs. hairs with up to 4 cells abundant); and the veins close up to the margin, with only 1–2 rows of cells between the vein tips and the margin (vs. submarginal, with (3–)4–8 rows of cells between the apex of the vein and the marginal cells). It also differs in the size and arrangement of the chlorenchyma cells of the lamina, of which some are elongated, with the main axis parallel to the vein (vs. isodiametric with cells not in rows parallel to the vein), and in the cells of the chlorenchyma and the sori, that in *H. cordobense* have abundant intercellular spaces (vs. intercellular spaces absent).

The morphological and anatomical differences of both taxa are shown in Fig. 2 and summarized in Tab. 1 and 2.

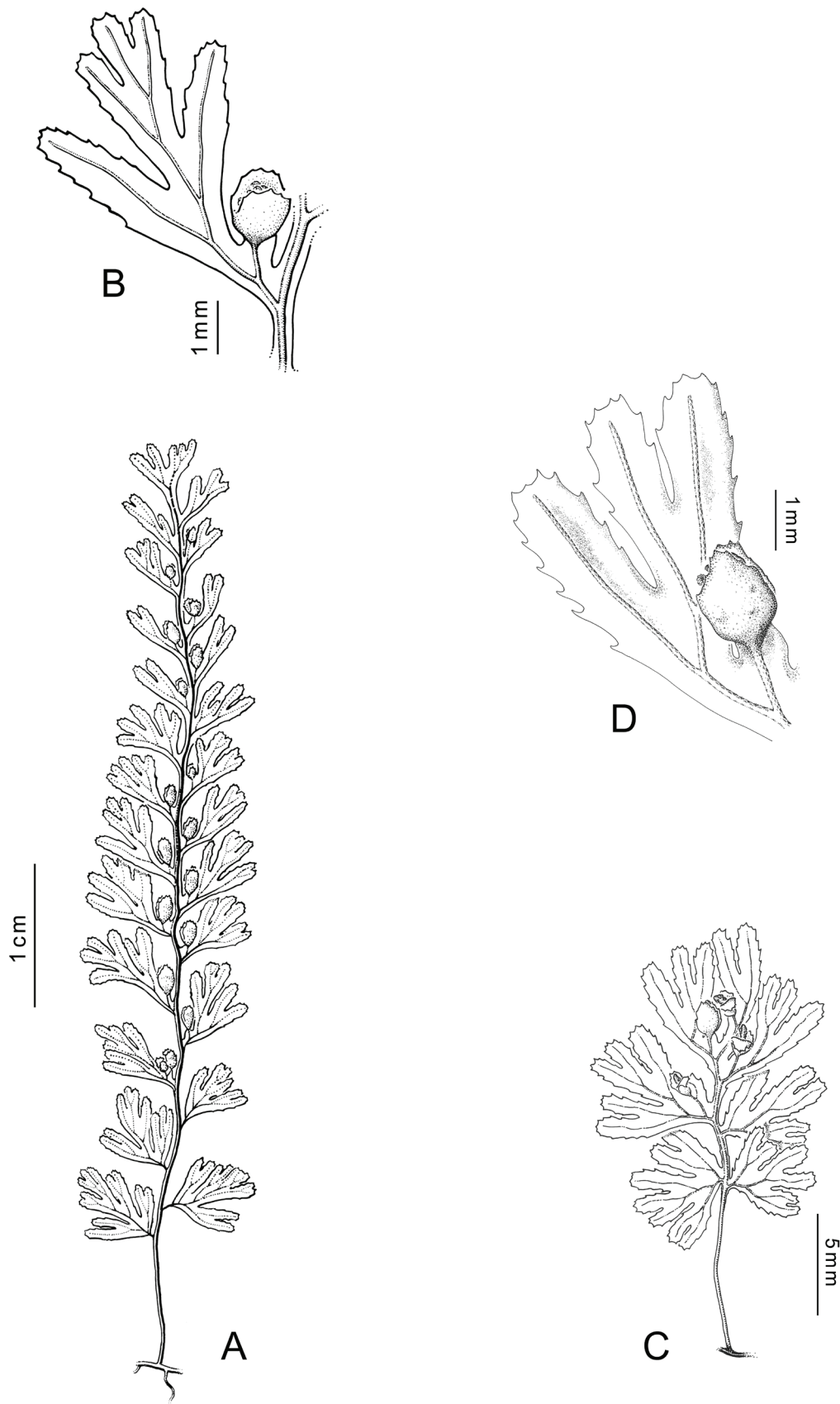


FIGURE 1. A, B. *Hymenophyllum cordobense*. A. Habit. B. Detail of a pinna with sori (Larsen160, SI). C, D. *H. asperulum*. C. Habit. D. Detail of a pinna with sori (Teillier s.n., CONC 143.373). Marcelo Moreno del.

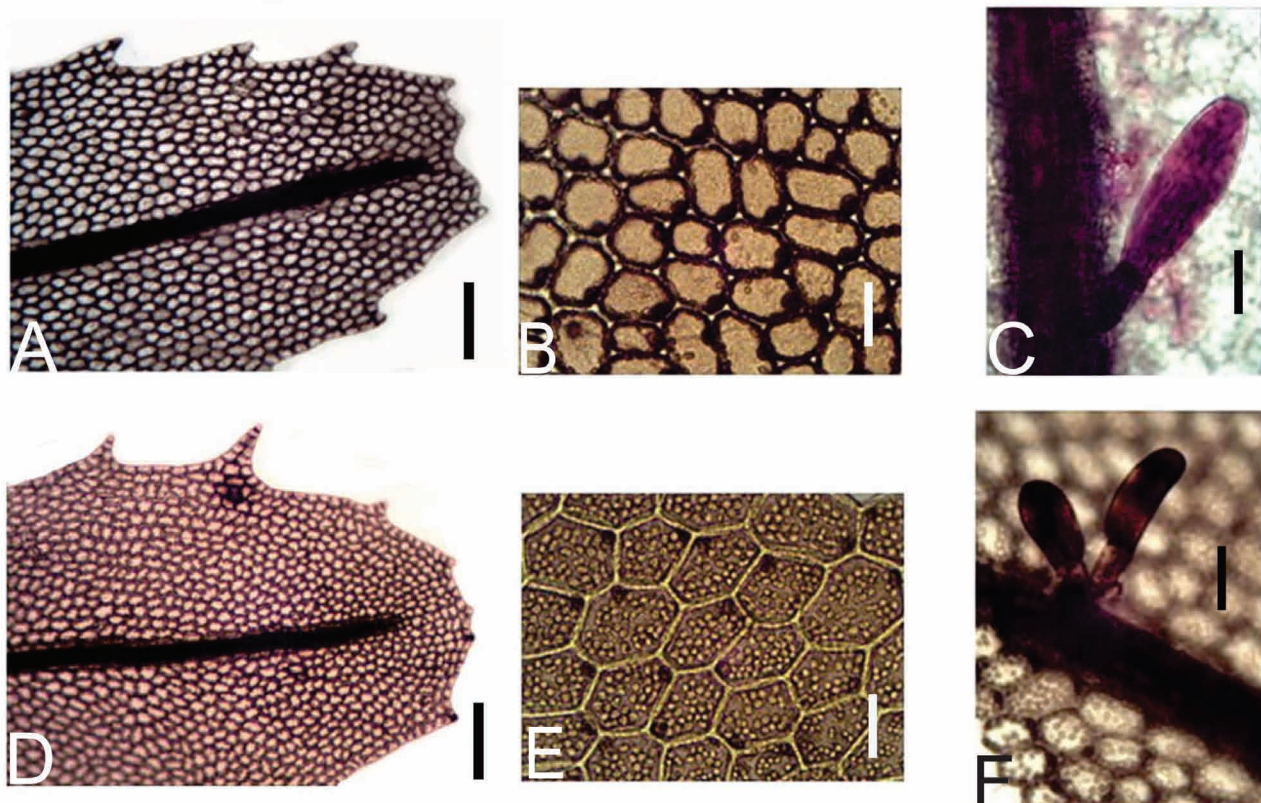


FIGURE 2. *H. cordobense*. **A.** Detail margin and apex of ultimate segment. **B.** Detail of cells of the chlorenchyma. **C.** Detail of a bicellular hair. (*Arana 726, RCVC*). *H. asperulum*. **D.** Details margin and apex of ultimate segments. **E.** Detail of cells of the chlorenchyma. (*Larsen 164, SI*). **F.** Detail of a bicellular hair. Scale bars: A and D= 150 μ m; B and E = 70 μ m; C and F = 65 μ m.

TABLE 1. Morphological comparison of the three species considered.

| | <i>H. cordobense</i> | <i>H. asperulum</i> | <i>H. tunbrigense</i> |
|--|---|---|---|
| Lamina shape | Ovate-lanceolate to linear-lanceolate with attenuate apex | Ovate to linear-elliptic with obtuse apex | Ovate to lineal lanceolate with acute apex |
| Lamina width | 0.5–1.2 cm | 0.8–1.7(–2) cm | 1.1–2.7 mm |
| Rachis | Alate from 4 th or 7 th pair of pinnae | Alate throughout | Alate throughout |
| Pinnae disposition | Remote, never imbricate | Proximate to imbricate, especially the apical pinnae | Proximate to imbricate |
| Segment width | 0.5–0.8 (–1) mm | 1.0–1.2 mm | 0.7–0.9 (–1.2) mm |
| Segment tips | Truncate to obtuse | Acute to obtuse, rarely truncate | Rounded |
| Sori | Ovate, with acute base slightly thickened, not immersed in the lamina, and rounded entire to erose or somewhat denticulate apex | Rhombic, with acute base slightly thickened, partly immersed in the lamina, and entire to erose or irregularly denticulate apex | Obovate to elliptic, with thickened, partially immersed acute base, and rounded erose or irregularly dentate apex |
| Hairs on rachis and veins | Short bicellular capitate hairs, rarely longer simple hairs | Bicellular, capitate hairs on veins and simple ones with 2–4 cells, with acute apices on rachis and petiole | Short, capitate, with 2–3 cells and simple ones with 3–6 cells, acute apices on the underside of rachis and veins |
| Capitate hairs | With the apical cell ellipsoid, 2–3 times longer than wide | The apical cell spherical, obovoid or cylindrical | The apical cell ellipsoid or reniform |
| Cells between the apex of the vein and the margin of the segment | 1–2 rows | (3–)4–8 rows | 2–10 rows |

TABLE 2. Anatomical comparison between the two southern South American species.

| | <i>H. cordobense</i> | <i>H. asperulum</i> |
|---|------------------------------|---------------------|
| Chlorenchyma cells of the segments | Elongated | Isodiametric |
| Cell orientation in the segments | In rows parallel to the vein | Not in rows |
| Intercellular spaces in chlorenchyma and sori | Always present | Absent |

Molecular phylogeny

The total alignment length of the four markers was 5297 bp (1207 *rbcL*, 1508 *rbcL-accD*, 998 *rps4-trnS*, 1101 *trnG-R*), with a total of 543 parsimony informative sites.

In the Bayesian analysis, all the *H. tunbrigense* specimens from Europe, Africa and newly acquired from South America are recovered in the strongly supported (posterior probability [PP]=1) “*H. tunbrigense* clade” (Hennequin *et al.* 2010, fig. 4, grey square) with the Chilean *H. rugosum* as a sister species (Fig. 3). Additionally, the South American specimens form a clade with strong support (PP=0.97) separated from the specimens of Europe and Tanzania, which are grouped in a strongly supported sister clade (PP=0.90). It is important to note that in the South American clade, our results show two highly supported subclades. The specimens from central and northern Argentina (i.e. those from Córdoba, Jujuy and Tucumán provinces) form one subclade (PP=1), and are separated from the specimens of the Patagonian forests of Argentina and Chile (PP=0.99).

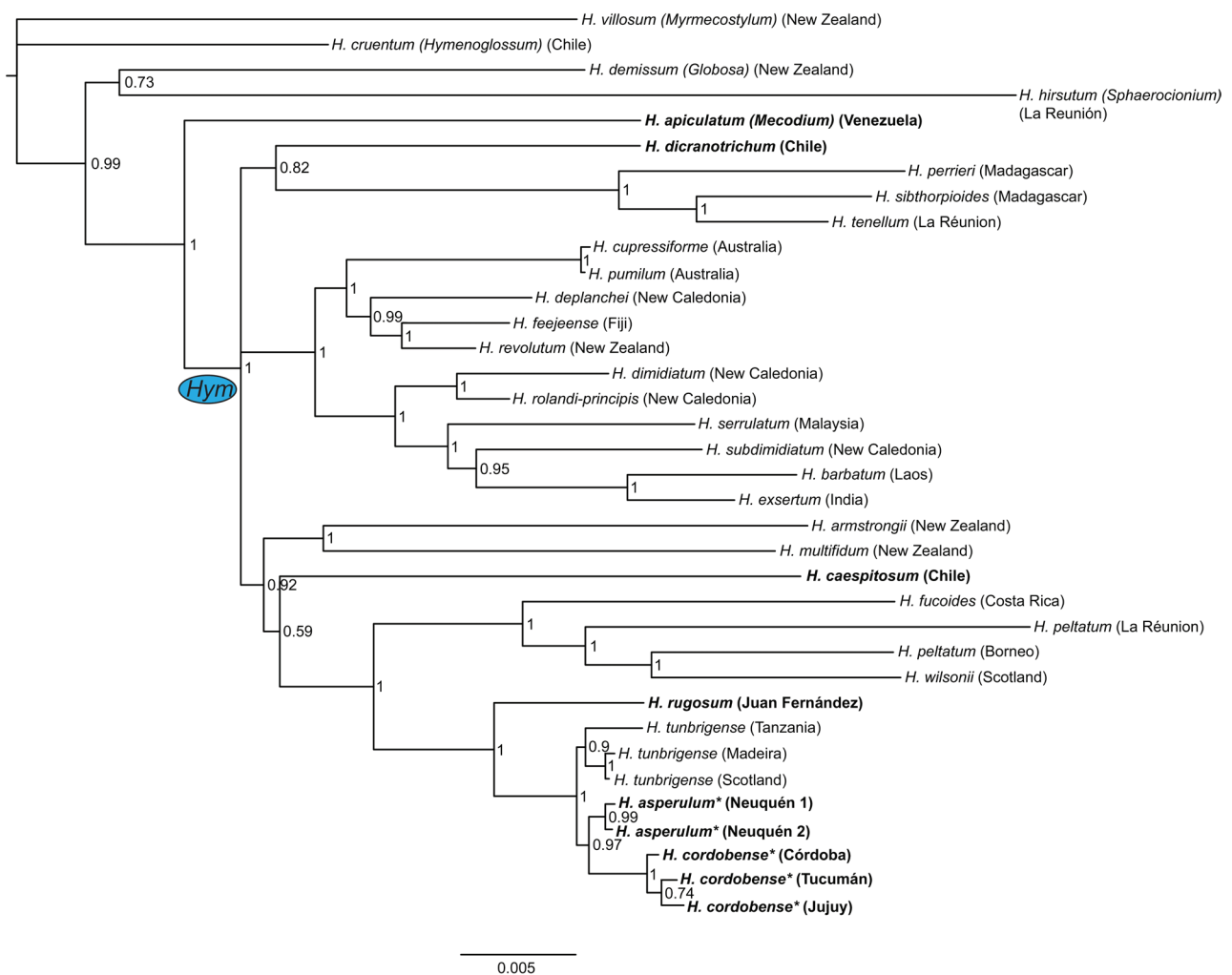


FIGURE 3. Bayesian 50% majority-rule consensus tree from 15,002 trees generated by Bayesian inference with MrBayes in the phylogenetic analysis of *Hymenophyllum* subgenera using the combined *rbcL*, *rbcL-accD*, *rps4-trnS* and *trnG-R* dataset. The values in nodes correspond to the Bayesian posterior probability (PP) support. The specimens identified with an asterisk (*) were sequenced for this work. The blue oval indicates the clade that correspond to the subg. *Hymenophyllum*. The localities are indicated in brackets as the same as the subgenera of outgroup specimens.



FIGURE 4. **A.** Habitat of *H. cordobense* in hyper humid place of Southern Andean Yungas. **B.** Habitat of *H. cordobense*: Rock shelters in highland grasslands of Chacoan Pampean Mountain. **C.** *In situ* population in the rock shelters in type locality, Sierra de Achala, Córdoba mountains in central Argentina, where *H. cordobense* can locally form dense populations. **D.** *In situ* population of *H. asperulum* in the Patagonian forest, Argentina.

Ecology

The European species of the “*Hymenophyllum tunbrigense*” clade grows on moist, shady rocks and tree trunks among bryophytes, usually in shady and humid places (Rich & Rumsey 2004), and most of its present or former localities in Europe are below 300 m (Richard & Evans, 1972). The species *H. asperulum* from Argentina and Chile is terrestrial, epiphytic and saxicolous, mat-forming, growing tightly adpressed on the substrate, especially on trunk bases in humid forests, or near watercourses, among bryophytes, usually in shaded places at lower locations (Larsen *et al.* 2013; Larsen, 2014), and has been collected between 0–800 m. On the other hand, *H. cordobense* grows in rock crevices of hyper humid habitats of caves and rock shelters in the wettest places from 1200 to 3750 m elevation in northern Bolivia (Figs. 4 A–D); accompanied by other *Hymenophyllum* species as *H. crispum* Kunth in Humboldt, Bonpland & Kunth (1816: 26) and *H. capurroi* de la Sota (1972: 54), and by other species of ferns as *Elaphoglossum piloselloides* (Presl 1825: 14) Moore (1857: 13), *Elaphoglossum hybridum* (Bory 1804: 95) Brackenridge (1854: 69) and *Melpomene peruviana* (Desvaux 1827: 231) Smith & Moran (1992: 430) (Arana 2012, Ganem *et al.* 2013). Although de la Sota (1972, 1973, 1977) pointed out that *H. cordobense* is epiphytic, we have observed this ecology neither in the Chaco Serrano (Córdoba) nor in the Yungas (Catamarca, Jujuy, Salta and Tucumán), this condition being likely extremely rare for the species.

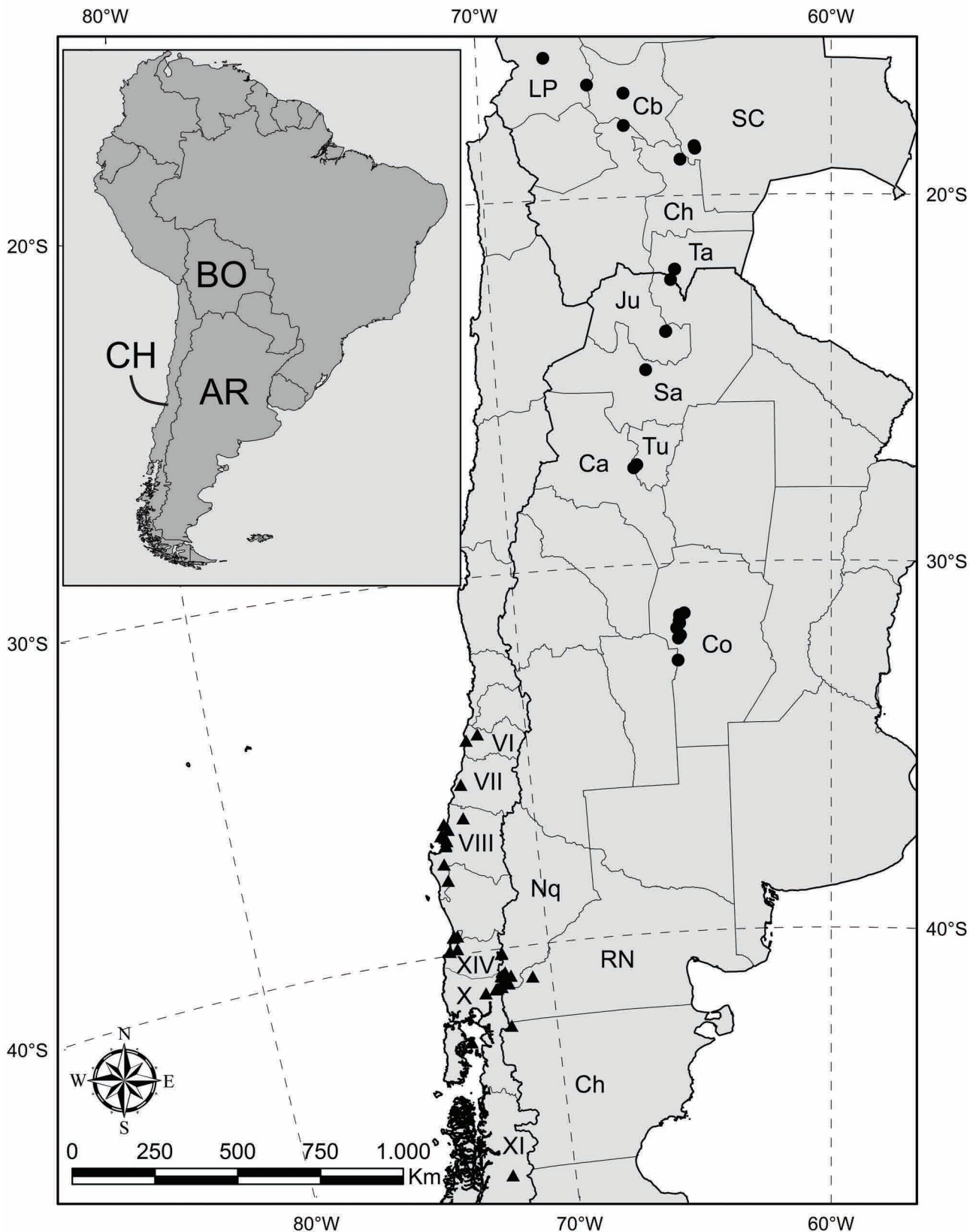


FIGURE 5. Distribution map of both species: *H. asperulum* (triangles), and *H. cordobense* (circles). AR: Argentina. CH: Chile. BO: Bolivia. **Argentine provinces.** Ca: Catamarca. Co: Córdoba. Ju: Jujuy. Sa: Salta. Tu: Tucumán. Nq: Neuquén. RN: Río Negro. Ch: Chubut. **Bolivian departments.** Cb: Cochabamba. Ch: Chuquisaca. LP: La Paz. SC: Santa Cruz. Ta: Tarija. **Chilean regions.** VI: O'Higgins. VII: Maule. VIII: BíoBío. XIV: Los Ríos. X: Los Lagos. XI: Aisén.

Distribution

Hymenophyllum tunbrigense occurs in the Azores, England, Ireland, Scotland, with scattered populations in the Canaries, Madeira, Spain (Galicia, Basque country), France (especially Normandy and Brittany), Belgium, Germany, Luxembourg, Italy, the Czech Republic, northeast Turkey and the east shore of the Black Sea (Adzharya) (Rich & Rumsey 2004). It has also been reported from North America (Farrar 1993), Central America and the Caribbean (Mickel & Smith 2004) however specimens of these areas have not been included in this analysis. In the current treatment given here, *H. tunbrigense* is excluded from Argentina and Chile.

Hymenophyllum asperulum is restricted to the Magellanic and Valdivian forests of the Subantarctic sub-region of the Andean Region (Morrone 2006, 2015). In Argentina it inhabits Chubut, Neuquén, Río Negro and Santa Cruz provinces, and is found in Chile from near Pichilemu (Libertador General Bernardo O'Higgins Region) to Coihaique (Aisén del General Carlos Ibáñez del Campo Region), with a few collections in Juan Fernandez Islands (Diem & Lichtenstein, 1959) (Fig. 5).

Hymenophyllum cordobense was currently known as endemic of the mountainous area of central and north-western Argentina (Arana 2012, Arana *et al.* 2016, Larsen 2014) and here is cited for the first time for the Bolivian flora. In southern South America, *H. cordobense* is found in forest clearings and at the edges of the Yungas in north-western Argentina, Bolivia and in the highland grasslands in central Argentina (Fig. 5).

Hymenophyllum cordobense and *H. asperulum*, both southern South American species, biogeographically are separated by the South American transition zone (SATZ, Morrone 2006). This area was greatly influenced by the geological history of the Andes, during their final and major uplift toward the end of the Pliocene and also the Pleistocene glaciations (Taylor 1991, 1995, Gregory-Wodzicki 2000), which resulted in the current aridification of SATZ, process that began in the Miocene (25.5 millions of years ago). Such events may have affected the evolution of Hymenophyllaceae in Argentina, since they caused fragmentation and differentiation of biotas with relatively young, pronounced vicariance events, especially those with the highest rainfalls (Arana *et al.* 2016), and could explain the distinctiveness of these two closely related species.

Conclusion

This study showed that there are significant morphological, anatomical and molecular differences between *Hymenophyllum cordobense* and *H. asperulum* and both of them from *H. tunbrigense*. The anatomical features (concerning lamina and sori) are highlighted as new and reliable diagnostic characters. Indeed, the taxa may be further distinguished based on cell shape of chlorenchymatous tissue of the lamina and the cells that form the tissue of the sorus. Besides the arrangement of these cells in the tissues of the plant, the features of the bicellular hairs on rachis and veins can be considered. Overall, given the quite clear geographical and ecological separation between *H. cordobense* and *H. asperulum*, both taxa represent separate lineages, retaining their genetic and phenotypic integrity. Thus, we deem it appropriate to apply the name *H. asperulum* to the specimens from Patagonian region and to give species rank to *H. cordobense*. This decision is important in the context of the conservation of the species given the habitat loss. More studies are necessary to confirm the taxonomic identity of North and Central American species.

Taxonomic treatment

Key to the species of the *Hymenophyllum tunbrigense* clade

1. Plants robust, fronds more than 10 cm long, laminae 3–4-pinnatifid, indusia notably dentate, rarely entire margin; plants from Central America, North America, Europe and Africa. *H. tunbrigense*
- Plants tenuous, fronds up to 8.5 cm long, laminae 2–3-pinnatifid, indusia entire to slightly denticulate or erose margin; plants from southern South America. 2
2. Fronds generally dense, with imbricate pinnae; plants from temperate forests from southern Argentina and Chile ... *H. asperulum*
- Fronds generally elongate, with spaced pinnae that never overlap; plants from subtropical forests and wet mountainous microhabitats from central-northern Argentina and Southern Bolivia. *H. cordobense*

Hymenophyllum asperulum Kunze (1834: 109). Fig. 1 C–D

Hymenophyllum tunbrigense (L.) Sm. var. *asperulum* (Kunze) Diem & J.S. de Lichtenstein (1959: 717). TYPE:—Chile. Región del Bío-Bío: “Obtegit praerupta humida argillosa ad Talcahuano flor. Chilens.”, *E.F. Poeppig Diar. 148 [Herb. Kunze]* (holotype not located; isotype BR 0000006965808!, possible isotype P 00622168!).

Hymenophyllum tunbrigense auct., non (L.) Sm. (1793: 418).

Rhizomes filiform, divided, dark brown, 0.3–0.4 mm in diameter, bearing scattered reddish trichomes 0.25–0.65 mm long, and small, delicate roots; fronds distant, spaced ca. 1 cm, 1.7–8.5 cm long; stipes dark brown, 0.6–3.5 cm, non-alate, with scarcely simple hairs; lamina ovate to linear, pinnate-pinnatifid to 2–3-pinnatifid, occasionally with reddish hairs on veins and at the base of indusium, to 4 cells; rachises dark brown, straight or in zig-zag, alate throughout, widest at the apex, or only alate at the apex, hairy or not; pinnae ovate, elliptic to rhombic, 2-pinnatifid, 0.5–1.2 × 0.3–0.8 cm, divided toward both sides, at least the basal ones, imbricated to some distant; ultimate segments linear to oblong, 1–1.2 mm width, margins with pluricellular teeth and rounded to truncate apex; sori 1 per pinna, subaxillar, on an acroscopic, short, narrowly alate segment, flexed away from the perpendicular plane of the lamina at maturity; indusia rounded to rhomboidal, with acute base, slightly immersed in the segment, apex rounded, the valves open half or more at maturity, with entire to denticulate or erose margins, occasionally with reddish hairs at the base, receptacle cylindrical inserted, shorter than the indusium.

Distribution and habitat:—*Hymenophyllum asperulum* grows mainly in northern region of the Patagonian forest of Argentina, and is common in Nahuel Huapi National Park. In Chile has been collected from near Pichilemu to near Coihaique, with a few collections from Juan Fernández Islands (Diem & Lichtenstein, 1959). It is terrestrial, epiphytic or saxicolous, and grows between the roots and in the base of the trees in wet forests, or near bodies of water, often mixed with mosses at 0–800 m (Fig. 4 D).

Representative specimens examined:—ARGENTINA. **Chubut:** Depto. Cushamen, Parque Nacional Lago Puelo, sendero a Gendarmería, 42.093°S, 071.649°W, 270 m, 07 December 2009, *Biganzoli et al. 2421* (SI). **Neuquén:** Depto. Lácar, Huahum, cascada Chachín, 40.141°S, 071.670°W, 750 m, 31 March 1952, *Diem 2119* (SI); Huahum, 40.120°S, 071.656°W, 30 March 1952, *Diem 2113* (SI); Depto. Los Lagos, Península Quetrihué, lago Huahuan, 14 April 1943, *Diem 603a* (SI); Brazo Rincón, Península Panguinal, Cerca del camping, cruzando el río Totoral, orilla del lago, 40.727°S, 071.776°W, 21 February 2011, *Larsen 164** (SI); Brazo Rincón, Península Panguinal, Cerca del camping, cruzando el río Totoral, orilla del lago, 40.728°S, 071.773°W, 21 February 2011, *Larsen 167** (SI); Arroyo Colorado, desembocadura, 40.850°S, 071.681°W, 12 May 1940, *Diem 572* (BAB); Península Quetrihué, 40.832°S, 071.627°W, 10 August 1941, *Diem s.n.* (BAB 91691); Lago Nahuel Huapi-Río Colorado, 40.850°S, 071.681°W, 770 m, 26 June 1951, *Diem 1920* (LIL); Península Quetrihué, Lago Nahuel Huapi, 40.832°S, 071.627°W, 700 m, 21 January 1951, *Sleumer 1542* (LIL); Quetrihué, arroyo desagüe Lago Escondido, costa del Lago Nahuel Huapi, 40.796°S, 071.646°W, 20 May 1944, *Diem 802* (SI; BAB); Quetrihué, cerca de la Punta Gaviota, 40.841°S, 071.601°W, 30 July 1942, *Diem 371* (SI; BAB); Quetrihué, costa Lago Huahuán, 40.824°S, 071.632°W, 08 July 1944, *Diem 851* (SI; BAB); Península Panguinal, Brazo Rincón, Lago Nahuel Huapi, 40.742°S, 071.748°W, 25 April 1943, *Diem 613* (SI; BAB); Quetrihué, costa oeste. Lago Nahuel Huapi, 40.835°S, 71.641°W, 14 August 1942, *Diem 381* (SI; BAB); Quetrihué, probablemente, 40.755°S, 071.441°W, 1945, *Diem s.n.* (SI 16533); Península Panguinal, Brazo Rincón, Lago Nahuel Huapi, 40.739°S, 071.752°W, 13 July 1941, *Diem 110 P* (SI; BAB); Quetrihué, Arroyo Corto, Costa Oeste, 40.864°S, 071.621°W, 31 July 1942, *Diem 373* (SI); Quetrihué, costa N.E., 40.643°S, 071.629°W, 04 June 1943, *Diem 647* (SI); Quetrihué, Punta Gaviota, 40.866°S, 071.618°W, 18 September 1949, *Diem 1196* (SI); Península Panguinal, Brazo Rincón, Lago Nahuel Huapi, 40.746°S, 071.741°W, 21 March 1943, *Diem 550* (SI); Quetrihué, orillas del Lago Huahuan, 40.826°S, 071.631°W, 26 July 1941, *Diem 111P* (SI); Quetrihué, Desagüe del Mallín Chico, 40.818°S, 071.620°W, 28 July 1943, *Diem 738* (SI); Lago Nahuel Huapi, Brazo Última Esperanza, cerca Puerto Barata, 40.713°S, 071.706°W, 770 m, 18 January 1948, *Diem 1112* (SI; BAB); Península Panguinal, Lago Nahuel Huapi en la costa, 40.745°S, 71.732°W, 04 October 1942, *Diem 400* (SI); Quetrihué, costa Oeste, 40.828°S, 71.643°W, 10 July 1941, *Diem 112P* (SI); Desembocadura Río Colorado, 40.850°S, 071.681°W, 12 May 1940, *Diem 113 P* (SI); Río Colorado, desembocadura, 40.852°S, 070.680°W, 23 June 1943, *Diem 729* (SI); Quetrihué sobre coste NE a orillas del Lago Nahuel Huapi, 40.803°S, 071.642°W, 06 November 1943, *Diem 741* (SI); Lago Nahuel Huapi, Brazo Última Esperanza, 40.706°S, 071.710°W, 04 July 1943, *Diem 736* (SI); Península Quetrihué, desagüe del Lago Patagua, 40.844°S, 071.617°W, 25 October 1941, *Diem 569* (SI, BAB); Lago Nahuel Huapi, Isla Victoria, Puerto Pampa, cerca del desagüe de un arroyo, 40.956°S, 071.546°W, 28 August 1942, *Diem 395* (SI, BAB). **Río Negro.** Depto. Bariloche, Puerto Blest, costa del Nahuel Huapi, 41.024°S, 071.811°W, 29 April 1942, *Diem 573^a* (SI); Puerto Blest, costa sur de la Bahía, 41.025°S, 71.814°W, 780 m, 22 October 1947, *Diem 1055* (SI, BAB). —CHILE. **VI Región de O'Higgins:** Prov. General Caro. Quebrada Los Robles, cerca de Pichilemu, 34.333°S, 071.950°W, 280 m, 12 October 1982, *Teiller s.n.* (CONC 143373); Quebrada Los Robles, 34.200°S, 071.570°W, 280 m, *Teiller s.n.* (CONC). **VII Región del Maule:** Prov. Cauquenes, Parque Nacional Los Ruiles, 35.817°S 072.500°W, 377 m, *Armesto et al. 992567* (CONC). **VIII Región del Biobío:** Prov. Concepción, Parque Hualpén, 36.788°S, 073.176°W, 21 April 1970, *de la Sota 6033* (LP); Concepción, 36.644°S, 072.909°W, 03 January 1966, *Meyer 9748* (LP); Concepción, Chiguayante, Cerro Manquimávida, 36.940°S, 073.001°W, 1 October 1938, *Barros 2238* (SI); Concepción, 36.845°S, 073.044°W,

25 October 1938, *Barros 2239* (SI); Concepción, 36.817°S, 073.050°W, 10 m, *Neger s.n.* (CONC); Ranquil, Fundo El Milagro, 36.650°S, 072.600°W, 100 m, *Sparre 10057* (CONC); Cerro Caracol, 36.833°S, 073.033°W, 200 m, *Behn s.n.* (CONC); Cerro Caracol, 36.833°S, 073.033°W, 200 m, *Ricardi s.n.* (CONC); Camino a Nonguen por la cantera, 36.833°S, 073.033°W, 130 m, *Pfister 452* (CONC); Pedro de Valdivia, 36.833°S, 073.050°W, 100 m, *Junge s.n.* (CONC); Cajón El Ciprés, interior de Colcura, 37.067°S, 073.083°W, 250 m, *Marticorena et al. 1057* (CONC); Cuesta de Colcura, 37.100°S, 073.117°W, 180 m, *Marticorena et al. 1103* (CONC); Quebrada Honda, Puente Mellizos, 37.117°S, 073.117°W, 175 m, *Marticorena et al. 1077* (CONC); Prov. Arauco, Lago Lanalhue, 37.917°S, 73.333°W, *Rodriguez R. 648* (CONC); Contulmo, Cerro Santa Elena, 38.000°S, 073.233°W, 30 m, *Ricardi s.n.* (CONC). **X Región de Los Lagos:** Prov. Llanquihue, 39.893°S, 073.434°W, 17 January 1909, *Hicken s.n.* (SI 18943); Llanquihue, 39.850°S, 073.167°W, 100 m, *Kubitzki 2* (CONC); Corral, cueva La Aguada, 39.879°S 073.436°W, September 1929, *Gunckel 1171.4* (SI); Lac Todos los Santos, 41.136°S, 072.401°W, February 1910, *Hauman 7* (LIL 40862); Llanquihue, 39.850°S, 073.167°W, 100 m, 1961, *Kubitzki 2* (CONC); Peulla, cascada de los helechos, 41.047°S, 071.962°W, 250 m, 28 October 1947, *Diem 1083* (SI; BAB); Peulla, 41.066°S, 72.029°W, 200 m, 26 October 1947, *Diem 1071* (SI; BAB); Chiloé, Isla Chaulinec, Capilla Vieja, 42.633°S 073.267°W, 25 m, *Villagrán & Aguila 6072* (CONC). **XIV Región de Los Ríos:** Prov. Valdivia, Gruta La Aguada, Corral, 39.883°S, 073.417°W, *Rudolph 572* (CONC). **XI Región Aisén del General Carlos Ibáñez del Campo:** Prov. Coihaique, El Sitio, Cerro El Cielo, 46.133°S, 072.700°W, 610 m, *Godoy et al. 63* (CONC).

Hymenophyllum cordobense (Hieronymus) C. Larsen & Arana, **stat. nov.** Fig. 1 A–B.

Hymenophyllum tunbrigense (L.) Sm. var. *cordobense* Hieronymus (1896: 361). Type:—ARGENTINA. Córdoba: Sierra de Achala, en la quebrada, cerca del Puesto Alegre, 5 March 1877, *G.H.E.W. Hieronymus 873* (lectotype CORD 00001407!, designated by Larsen & Ponce, *Flora Argentina* 2: 198. 2016; isolectotype SI 012004!).

Hymenophyllum wilsonii Hooker (1844: 95) var. *achalensis* Hieronymus (1896: 361). *Hymenophyllum peltatum* (Poirlet 1808: 76) Desvaux (1827: 333) var. *achalensis* (Hieronymus 1896: 361) Capurro (1940: 73). “Type:—ARGENTINA, Córdoba: na der Cuesta Del Gaucho, 24 February 1876, *G.H.E.W. Hieronymus 456* (lectotype CORD 00001408!, designated by Larsen & Ponce, *Flora Argentina* 2: 198. 2016; isolectotype US 00067710!).

Rhizomes filiform, dark brown, long-creeping, 0.14–0.30 mm in diameter, usually bearing scattered reddish trichomes 0.3–0.6 mm long, and small, delicate roots; fronds distant, monomorphic, 3.3–10.0 cm long; stipes thin, dark brown, 0.5–2.5 cm × 0.1 mm, non-alate, with scarce bicellular hairs, 0.30–0.46 mm long, with the apical cell ovoid, 2–3 times longer than the basal one; laminae ovate-lanceolate to linear-lanceolate, with acute apex and truncate base, pinnate-pinnatifid to 3–4-pinnate, 2.7–9.0 × 0.5–1.2 cm, sparsely pubescent on rachis and veins, the hairs similar as those of the stipes; rachises dark brown, alate, especially from the 4–7th pair of pinnae to the apex; pinnae flabellate to rhombic to trapeziform, distant, ascendant, 6–12 pairs per lamina, with few (2–4) segments, 3–4-pinnatifid, basal pinnae divided on both sides, the apical ones more developed in the acroscopic part; ultimate segments bearing a single vein, linear, 0.8–1.0 mm, with serrate margins and obtuse apex; venation pinnate and anadromous; sori paratactic, terminal on an acroscopic, short, narrowly alate segment, subaxillar, flexed away from the perpendicular plane of the lamina at maturity, 1 per pinna (rarely 2); indusia ovate, not immersed in the lamina, with a thickened base, distinctly bilabiate, the lips with entire to erose or denticulate margins, longer than half the length of the sorus; receptacle elongate, sometimes fusiform, not exserted, with ca. 30 sporangia.

Distribution and habitat:—*Hymenophyllum cordobense* grows in Central Andean rainforests in central and northwestern Bolivia, in the Departments of Cochabamba and La Paz. It is also present in the Tucuman-Bolivian-forest and Southern Andean Yungas of Southern Bolivia, in the Departments of Chuquisaca, Santa Cruz, and Tarija; and in Argentina, in the provinces of Catamarca, Jujuy, Salta and Tucumán. It also inhabits highland grasslands (“Pastizales de altura”) of Córdoba province in Argentina. This species grows in rock crevices of hyper humid habitats of caves and rock shelters in the wettest places from 1200 to 3,750 m (Figs. 4 A–C).

Representative specimens examined:—ARGENTINA. **Catamarca:** Depto. Andalgalá, Estancia Yunca Suma, 2400 m, 21 January 1949, *Petersen (Herb. Diem 1262)* (SI). **Córdoba:** Depto. Calamuchita, Villa Alpina, 11 October 2009, *Larsen 68* (SI); Depto. Cruz del Eje, Ruta Provincial 28, de Tanti a Tanninga, pie oriental de Los Gigantes, 1860 m, 08 October 2009, *Zuloaga 11314* (SI); Quebrada del Rodeo al pié de Los Gigantes, Sierra de Achala, *Hieronymus s.n.* (CORD 1405); Depto. Punilla, Los Gigantes, Sierras Grandes, 1858 m, 27 March 2011, *Morero 336* (CORD); Depto. Río Cuarto, Mesada de Crespo, pastizal de altura, *Bianco 2134* (RIOG); Depto. San Alberto, Pampa de Achala, *Morero 279* (SI); Ruta Provincial 20 de Mina Clavero a Córdoba, 1560 m, 09 October 2009, *Zuloaga 11323** (SI); Sierra de Achala, en la quebrada, cerca del Puerto Alegre, 05 March 1877, *Hieronymus 873* (SI); Cuesta del Gaucho,

Sierra de Achala, *Hieronymus 456* (CORD); Pendientes del Cerro Champaquí, más arriba del Río del Catre, 30 January 1877, *Hieronymus 778* (CORD); **Jujuy:** Depto. Ledesma, Parque Nacional Calilegua, Aguada del Tigre, 1630 m, 05 July 2010, *Larsen & Arana 160* (SI, RCVC); Parque Nacional Calilegua, descendiendo desde Monolito hacia Mesada de las Colmenas, 1616 m, 05 July 2010, *Larsen & Arana 140** (RCVC, SI); **Salta:** Depto. Capital, Quebrada de San Lorenzo, saxícola, 2100 m, 25 August 1985, *Palací 149* (MCNS); Depto. Santa Victoria, Los Toldos, sobre capa muscinal en tronco, 1600 m, 6 July 1998, *Martinez & de La Sota 674* (MCNS). **Tucumán:** Depto. Chicligasta, Casa de Piedra, afluyente del arroyo del Bajo Grande, 1233 m, 04 November 2009, *Larsen 107** (SI).—BOLIVIA. **Chuquisaca:** Prov. Belisario Boeto, ca. 20 km de Serrano camino hacia Nuevo Mundo, 2185 m, 13 May 2004, *Huaylla 1071* (LPB). **Cochabamba:** Prov. Ayopaya, 2 km al SE de Saila Pata, 3,750 m, 15 November 1997, *Kessler 12492* (LPB); Prov. Chapare Carretera nueva a Santa Cruz, pasando la represa de Corani, 3,240 m, 29 September 2002, *Jimenez 1744* (LPB). **La Paz:** Prov. Murillo, Valle del Río Zongo, 20 km al N de la cumbre, 3,400 m, 17 November 1987, *Solomon 17428* (LPB); Prov. Murillo, Valle del río Zongo. 20 km al N de la cumbre, 3,400 m, 17 November 1987, *Solomon 17428* (LPB). **Santa Cruz:** Prov. Valle Grande, 5 km de Loma Larga a Valle Grande, 2100 m, 07 June 1996, *Kessler 6351* (LPB); Prov. Florida, Cantón Mairana, Parque Nacional Amboro, 7 km NNE by air from Mairana, 2300 m, 22 July 1994, *Moran 5894* (LPB); Prov. Valle Grande, 13 km de Loma Larga a Valle Grande, 2300 m, 11 June 1996, *Kessler 6454* (LPB); 13 km de Loma Larga a Valle Grande, 2300 m, 11 June 1996, *Kessler 6485* (LPB). **Tarija:** Prov. Aniceto Arce Ruiz, Reserva Natural de Flora y Fauna Tariquía, campamento Alisos, 2220 m, 13 June 2004, *Jimenez 2421* (LPB); Reserva Natural de Flora y Fauna Tariquía, campamento Alisos, 1860 m, 09 June 2004, *Jimenez 2372* (LPB); Reserva Natural de Flora y Fauna Tariquía, campamento Alisos, 1860 m, 09 June 2004, *Jimenez 2374* (LPB).

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Appendix 1.

Hymenophyllum tunbrigense. **Specimens examined**:—FRANCE. **Finèstere**; Huelgoat, 20 October 1898, *Ménager s.n.* (SI 22751); Huelgoat, July 1961, *de Joncheere WEF 12* (SI). **Manche**, Cherbourg, July 1855, *de Schoenefeld s.n.* (SI 22750); Environs de Cherbourg, 31 may 1905 *Martin s.n.* (SI22752); Cherbourg, 20 July 1885, *Beaudoun s.n.* (SI 22749).—PORTUGAL. **Madeira**; Cabo de Pacegueiros, February 1962, *de Joncheere MAD 97* (SI); Santo da Serra, above Lamaceiros, *de Joncheere MAD 89* (SI). —SOUTHAFRICA. **Western Cape**; Swellendam, August 1965, *de Joncheere SAC 136* (SI); Kirstenbosch, Skeleton George, Capetown, March 1957, *de Joncheere SAC 83* (SI).

Appendix 2. GenBank accession numbers of *Hymenophyllum* taxa for chloroplast markers used in this study. GenBank accession numbers, with collector and herbarium acronym of voucher material sequenced in our work are in bold.

| Species | Specimen Voucher (herbarium acronym) | <i>rbcL</i> | <i>rbcL-accD</i> | <i>rps4-trnS</i> | <i>trnG-R</i> |
|--|--------------------------------------|-----------------|------------------|------------------|-----------------|
| <i>H. apiculatum</i> Mett. ex Kuhn | | AF275642 | AY775438 | AY095131 | GU200697 |
| <i>H. armstrongii</i> (Baker) Kirk | | AY095109 | AB162691 | AY095128 | GU200698 |
| <i>H. asperulum</i> Kunze | Larsen 167 (SI) | KY688276 | KY688281 | KY688286 | KY624598 |
| <i>H. asperulum</i> Kunze | Larsen 164 (SI) | KY688277 | KY688282 | KY688287 | KY624599 |
| <i>H. barbatum</i> (Bosch) Baker | | AB064287 | AB064299 | AY095124 | GU200699 |
| <i>H. caespitosum</i> Gaudich | | AF275649 | AB191456 | AY095130 | GU200700 |
| <i>H. cordobense</i> (Hieronymus) C. Larsen & Arana | Zuloaga 11323 (SI) | KY688278 | KY688283 | KY688288 | KY624600 |
| <i>H. cordobense</i> (Hieronymus) C. Larsen & Arana | Larsen 107 (SI) | KY688279 | KY688284 | KY688289 | KY624601 |
| <i>H. cordobense</i> (Hieronymus) C. Larsen & Arana | Larsen 140 (SI) | KY688280 | KY688285 | KY688290 | KY624602 |
| <i>H. cruentum</i> Cav. | | AY095107 | AB191455 | AY095133 | GU200701 |
| <i>H. cupressiforme</i> Labill. | | AB496560 | AB496560 | AB496582 | AB496600 |
| <i>H. demissum</i> (G. Forst.) Sw. | | AY775402 | AY775441 | AY775416 | GU200702 |
| <i>H. deplanchei</i> Mett. ex Kuhn | | AB064288 | AB064300 | AY095136 | GU200703 |
| <i>H. dicranotrichum</i> (C.Presl.) Sadeb. | | AB496562 | AB496562 | GU200682 | GU200704 |
| <i>H. dimidiatum</i> Mett. | | AB064289 | AB064301 | GU200683 | GU200707 |
| <i>H. exsertum</i> Wall. ex Hook. | | AB496567 | AB496567 | AB496588 | AB496604 |
| <i>H. feejeense</i> (Brack.) Copel. | | GU200663 | GU200672 | GU200684 | GU200708 |
| <i>H. fucooides</i> (Sw.) Sw. | | U20933 | AY775449 | AY095142 | GU200710 |
| <i>H. hirsutum</i> (L.) Sw. | | AY775407 | AY775450 | AY775432 | GU200712 |
| <i>H. multifidum</i> (G. Forst.) Sw. | | GU200664 | GU200673 | GU200686 | GU200717 |
| <i>H. peltatum</i> (Poir.) Desv. | | GU200665 | GU200674 | GU200687 | GU200718 |
| <i>H. peltatum</i> (Poir.) Desv. | | AB496564 | AB496564 | AB496585 | AB496601 |
| <i>H. perrieri</i> Tardieu | | AB496578 | AB496578 | AB496598 | GU200719 |
| <i>H. pumilum</i> Hook. & Baker | | AB496574 | AB496574 | AB496594 | AB496608 |
| <i>H. revolutum</i> Colenso | | GU200666 | GU200675 | GU200690 | GU200722 |
| <i>H. rolandi-principis</i> Rosenst. | | AY095110 | AB083286 | AY095143 | GU200723 |
| <i>H. rugosum</i> C. Chr. & Skottsb. | | GU200667 | GU200676 | GU200691 | GU200724 |
| <i>H. serrulatum</i> (C. Presl) C.Chr. | | AB496565 | AB496565 | AB496586 | AB496602 |
| <i>H. sibthorpioides</i> (Bory ex Willd.) Mett. | | AY095117 | AB162688 | AY095127 | GU200726 |
| <i>H. subdimidiatum</i> Rosenst. | | AB064290 | AB064302 | AY095140 | GU200727 |
| <i>H. tenellum</i> (Jacq.) Kuhn. | | AY095116 | AB191453 | AY095126 | GU200728 |
| <i>H. tunbrigense</i> (L.) Sm. | | GU200670 | GU200679 | GU200694 | GU200731 |
| | | GU200671 | GU200680 | GU200695 | GU200732 |
| | | AB496576 | AB496576 | AB496596 | GU200733 |
| <i>H. villosum</i> Colenso | | AB191454 | AB191454 | AY775429 | GU214753 |
| <i>H. wilsonii</i> Hook. | | GU200669 | GU200679 | GU200693 | GU200729 |